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CALCIUM AND PROTEIN CHANGES
IN SERUM DURING SLEEP AND
REST WITHOUT SLEEP

A PART OF A DISSERTATION SUBMITTED TO
THE FACULTY OF THE DIVISION OF THE
BIOLOGICAL SCIENCES IN CANDIDACY FOR
THE DEGREE OF DOCTOR OF PHILOSOPHY

DEPARTMENT OF PHYSIOLOGY

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By
NORMAN ROY COOPERMAN

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CALCIUM AND PROTEIN CHANGES IN SERUM DURING SLEEP AND REST WITHOUT SLEEP

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It has been frequently reported that there is a change in the total serum calcium during sleep and anesthesia. In 1924, Cloetta and Thomann (3) reported a drop in serum calcium during anesthesia. One year later, Heilig and Hoff (8), using psychopathic patients, demonstrated an increase during sleep, while Demole (4) found a decrease. Gollwitzer-Meier (7) was not able to demonstrate any significant change under this condition.

On the basis of his work with Thomann as well as that done by others, Cloetta postulated a passage of calcium from the blood stream into the tissues, including the brain, during sleep and anesthesia. This view was supported by Demole and Fischer (5, 6) but refuted by Bergren and Moberg (1) who obtained a state resembling sleep by the injection of calcium chloride, potassium chloride, and on occasion, by merely inserting the syringe needle into the appropriate region. In 1929 Cloetta and Fischer (2) repeated the work of Bergren and Moberg using a great number of substances. They corroborated Demole insofar as CaCl_2 injections were concerned, and found that KCl caused an excitation. They also reported that injections into adjacent regions had no effect and maintained that the result was specific for calcium in a delimited area of the brain.

All previous investigators had only measured changes in total serum calcium, and, as indicated above, their findings were not consistent. This investigation was undertaken to re-determine whether any changes in blood calcium actually occurred during sleep, with special reference to ionized calcium.

PROCEDURE. Human subjects and dogs were used in this study. Preceding each experiment, no food was taken for 12 and 24 hours respectively. In the human subjects, blood samples were taken before going to sleep and immediately upon awakening. These observations were controlled by having the same individuals lie awake without actually sleeping for an equal length of time. In the animal experimentation an exact criterion of sleep was lacking although the conditions were conducive to sleep. Hence, reference is made to these periods as "rest." The exercise preceding one

series of experiments was carried out by means of a treadmill operating at a very slow speed.

The chemical methods used were as follows: Clark-Collip modification of the Kramer-Tisdall for total serum calcium; macro-Kjeldahl for serum proteins; McLean-Hastings nomogram method for ionized calcium (10); and the Congo red indicator method for circulating blood volume.

RESULTS. In 7 subjects, on 7 experimental nights, 5 to 7 hours of sleep were found to cause a slight decrease in total serum calcium which, coupled with a greater decrease in serum proteins, resulted in a slight but consistent increase in the calcium ion concentration of the serum. Similar

TABLE 1
Calcium and protein changes in serum during sleep and rest without sleep

		HUMAN SUBJECTS			DOGS		
		I Effects of sleep (5-7 hours)	II Effects of lying awake (5-7 hours)	III Effect of sleep and rest without sleep (1½-2 hours)	IV Effect of rest (1-2 hours) after mild exercise (30-60 minutes)	V Effect of rest (1-2 hours) without initial exercise	VI Effect of prolonged rest (5-6 hours)
Grams per cent serum proteins	Normal	7.79 (7.17-8.39)	7.77 (6.81-8.58)	7.79 (6.97-8.84)	6.12 (5.40-6.51)	6.43 (5.76-7.35)	6.82 (6.44-7.35)
	Experimental	7.25 (6.64-7.58)	7.10 (6.31-7.87)	7.10 (6.13-8.30)	5.76 (5.14-6.21)	6.25 (5.00-7.14)	6.19 (5.84-6.65)
	% difference	-6.7	-8.6	-8.8	-5.7	-2.8	-9.2
Mgm. Ca per 100 cc. serum	Normal	10.11 (9.74-10.49)	10.05 (9.32-10.59)	10.50 (9.55-10.99)	10.68 (10.27-10.98)	10.47 (9.74-11.41)	10.52 (9.35-11.39)
	Experimental	9.87 (9.43-10.36)	9.98 (9.35-10.38)	10.10 (9.04-10.81)	10.50 (9.95-11.27)	10.49 (9.52-11.21)	10.16 (9.90-10.36)
	% difference	-2.3	-0.6	-4.6	-1.8	+0.5	-3.1
mM Ca ⁺⁺ per kgm. H ₂ O	Normal	1.11 (1.07-1.16)	1.11 (1.00-1.22)	1.18 (1.10-1.28)	1.38 (1.30-1.46)	1.32 (1.14-1.52)	1.27 (1.14-1.36)
	Experimental	1.16 (1.10-1.19)	1.18 (1.08-1.26)	1.18 (1.09-1.36)	1.39 (1.29-1.48)	1.35 (1.17-1.61)	1.31 (1.21-1.37)
	% difference	+4.0	+6.0	+0.1	+0.9	+2.1	+3.0

changes were found in the same subjects after they had remained awake in a recumbent position for an equal length of time. With a shorter period (1½ to 2 hours) of both sleep and rest without sleep, there was, in 11 experiments, a more marked decrease in total serum calcium, a decrease in serum proteins no greater than with an interval of 5 to 7 hours, and no change in the resultant calcium ion concentration. No differences were found when the experimental periods occurred in the afternoon.

One and one-half to 2 hours after the beginning of the experimental period there was a 10 per cent increase in plasma volume in both sleep and rest. At this time, red blood cell, hemoglobin and hematocrit determinations showed a decrease.

Ten experiments, using 5 animals, showed the effects of $1\frac{1}{2}$ to 2 hours of rest after a 30 to 60 minute period of mild exercise to be a small decrease in both serum proteins and total serum calcium, with no change in the calcium ion concentration. Without the initial exercise period, no consistency was seen in the changes in serum proteins and serum calcium obtained after rest, in 13 experiments using 5 dogs. However, where an increase in serum proteins was found, it was accompanied by an increase in total serum calcium, and vice versa. Under these conditions, calcium ion concentration showed no change. When the animals (4 dogs, 6 expts.) rested for a longer period (5 to 6 hrs.), there was found a decrease in both serum proteins and serum calcium with, however, a small, but consistent, increase in the calcium ion concentration. These results are based on the numerical values summarized in table 1.

DISCUSSION. The results suggest that the decrease in both total serum calcium and serum proteins occurring after sleep as well as rest can be accounted for by the increase in plasma volume, i.e., the passage of tissue fluids into the vascular system. This presumption is strengthened by a decrease in the other blood constituents, namely, erythrocytes, hemoglobin and packed red cells after $1\frac{1}{2}$ to 2 hours of rest, and by the increase in the Na and Cl content of the plasma known to occur during sleep. For lymph, and presumably intercellular fluid, contains a greater percentage of Na and Cl than does blood.

Katznelbogen (9) was of the opinion that if a transfer of calcium did take place, the calcium content of the brain should be greater in the sleeping than in the waking state. However, he found no difference in the whole brain calcium between anesthetized (diallyl-barbituric acid) and control animals. Because small changes in an isolated region may have been masked by the large bulk of brain, he repeated the procedure dividing the brain into various portions and comparing the calcium content of each. Here too, no significant difference between the two groups was observed; although he did find that, in both groups, the calcium content of the hypothalamic region was greater than that of other parts of the brain. He suggested some relation between this and hypothalamic activity. Katznelbogen's results refute Cloetta's view that the drop in blood calcium observed after sleep is to be explained by a transfer of calcium from the blood to the brain.

This decrease in total calcium can be explained on the basis of dilution by tissue fluids as suggested above. The same mechanism accounts for the fact that ionic calcium remains the same, for tissue fluids contain the same amount of ionic calcium as does blood. The calcium of tissue fluids is in equilibrium with the diffusible calcium of the blood, which McLean and Hastings (11) have shown to be almost entirely in the ionic state. The transfer of fluid explains the absence of a change in ionic calcium in the shorter periods of sleep or rest, but does not explain the increased

calcium ion concentration of the blood after the longer periods of sleep (or rest). It is apparent, however, that the *total amount of calcium* in the blood stream is greater after sleep (or rest) than before, as was previously supposed.

Our results confirm those of Gollwitzer-Meier and Thompson (15) as to hemodilution during rest. This can be considered a result of the decreased blood pressure obtaining during sleep (or rest). A decreased filtration pressure at the arterial end of the capillary bed in the presence of an adequate reabsorption at the venous end by virtue of the serum proteins, would tend to allow fluid to enter the bloodstream, thus increasing the circulating blood volume.

CONCLUSIONS

1. During sleep (5 to 7 hours) both total serum calcium and serum proteins decrease, with a resultant slight but consistent increase in the calcium ion concentration of the serum.

2. During rest without sleep for the same period, similar changes occur.

3. During sleep (and rest) for shorter periods ($1\frac{1}{2}$ to 2 hours) there is a more marked decrease in total serum calcium, but the ionized calcium remains the same.

4. The changes found in the shorter periods of sleep and rest can be correlated with an increase in circulating plasma volume.

5. These results disagree with Cloetta's view that during sleep calcium passes from the blood stream into the tissues.

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REFERENCES

- (1) BERGREN AND MOBERG. *Acta Psychiat. et Neurol.* **4**: 1, 1929.
- (2) CLOETTA AND FISCHER. *Arch. f. Exper. Path. u. Pharmacol.* **174**: 589, 1929.
- (3) CLOETTA AND THOMANN. *Arch. f. Exper. Path. u. Pharmacol.* **103**: 260, 1924.
- (4) DEMOLE. *Arch. f. Exper. Path. u. Pharmacol.* **120**: 229, 1927.
- (5) FISCHER. *Arch. f. Exper. Path. u. Pharmacol.* **133**: 169, 1928.
- (6) FISCHER. *Arch. f. Exper. Path. u. Pharmacol.* **153**: 254, 1928.
- (7) GOLLWITZER-MEIER AND KROETZ. *Biochem. Ztschr.* **154**: 82, 1924.
- (8) HEILIG AND HOFF. *Klin. Wehnschr.* **4**: 2194, 1925.
- (9) KATZENELBOGEN. *Arch. Neurol. and Psychiat.* **23**: 405, 1932.
- (10) MCLEAN AND HASTINGS. *J. Biol. Chem.* **107**: 337, 1934.
- (11) MCLEAN AND HASTINGS. *J. Biol. Chem.* **108**: 285, 1935.
- (12) MCLEAN AND HASTINGS. *Am. J. Med. Sci.* **189**: 601, 1935.
- (13) ROBERTS AND CRANDALL. *This Journal* **106**: 423, 1933.
- (14) ROWE. *J. Lab. and Clin. Med.* **1**: 485, 1915.
- (15) THOMPSON AND THOMPSON. *Proc. Nat. Acad. Sci.* **14**: 94, 1928.

